DEVELOPMENTAL CHANGES OF PIECES OF FROG EMBRYOS CULTIVATED IN LYMPH.

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In the course of some experiments on the behavior of the epithelial tissue of frog embryos when cultivated in lymph or plasma it was found that pieces of tissue sometimes underwent developmental changes. During the comparatively short time in which material was available for experimental work, opportunity was not found for devoting as much attention to the changes in these pieces as was desirable. Nevertheless a few of the things observed were sufficiently striking and suggestive to be worthy of record, and it is hoped that the observations may be made more complete at a future date.

The material used consisted of embryos and young larvæ of *Rana* taken out of the jelly a short time before they were ready to make their natural exit. The jelly was placed for a few minutes in an antiseptic solution, and the embryos were afterward cut to pieces in sterile Ringer's solution. Each piece was then mounted in a hanging drop of lymph or plasma of the adult frog, sealed up in a hollow slide, and kept in a cool place. Every few days the piece was transferred to a fresh medium.

Changes in the ectodermic epithelium were quite manifest a few hours after the pieces were mounted, and in one or two days remarkable strands and sheets of ectodermic cells were to be seen extending into the culture medium. These structures and the behavior of their component cells are more fully described in another paper now in course of publication. But aside from the movements of the ectoderm, there were, in certain of the pieces, marked developmental changes of the internal parts. Irregular fragments of tissue frequently became more rounded in form; sometimes they put out lobes or processes of various kinds, and, in some cases, they increased noticeably in size. In a piece from the head region of a late embryo shortly before the rudiments of gills made their appearance, there were developed,

in addition to several larger lobes, a number of finger-like processes which very closely resembled the filaments of the gills. The central part of these projections was composed of connective tissue, and the epithelial covering consisted of a layer of columnar

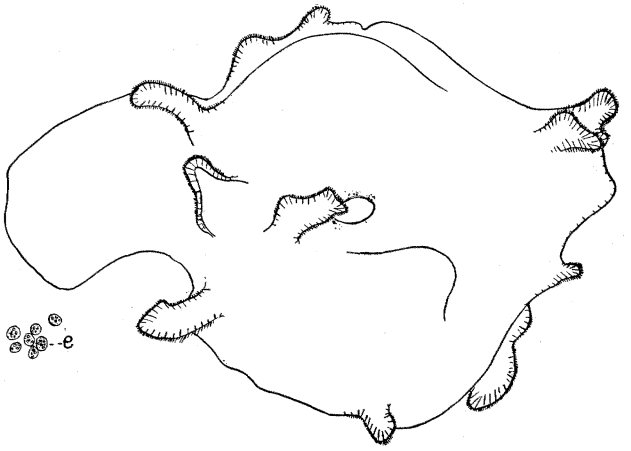

FIG. 1. Piece from the head of a frog embryo drawn fifteen days after being cultivated in lymph. *e*, isolated ectoderm cells.

ciliated cells. These outgrowths began three days after the preparation was made, and they were practically completely formed in a week. Fifteen days after the piece was mounted it had assumed the form shown in Fig. 1. The cilia on the projections were beating, and in fact they continued active for two more weeks, but there was little further change in the general form of the piece. When originally mounted the piece was opaque, and the cells composing it contained a large amount of yolk. After two weeks it had become nearly transparent; the larger part of the yolk in the cells had been assimilated, and a typical connective tissue had developed in the interior from the comparatively unspecialized cells of the mesoderm. The piece was covered completely by ectoderm which, over most of the surface, was flattened, but assumed a cylindrical form on the more prominent projections. In the third week a layer of epithelium was sloughed off from a large part of the surface of the piece.

Whether the finger-like processes represent gill filaments is more or less open to question. Processes of this kind failed to make their appearance from the hinder part of the embryos.

Should they be gill filaments they would afford a case of the self differentiation of an organ in the absence of certain stimuli, such as those afforded by the blood supply, which normally are associated with their formation.

Fig. 2 represents a development from a cross section of a late embryo near the middle of the tail region. This piece, like the one just described, was at first opaque, owing to the large amount of yolk in the cells. A week later it became quite transparent,

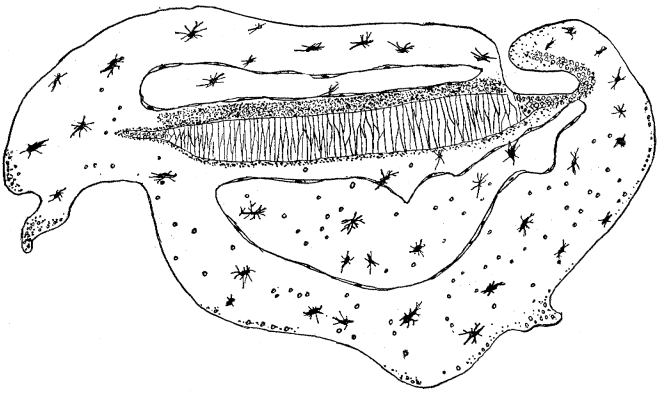

FIG. 2. A cross section from near the middle of the tail of a frog embryo drawn seven days after being cultivated in lymph.

and numerous pigment cells became differentiated beneath the ectoderm. Externally it was completely covered by a layer of flattened epithelial cells. The connective tissue within the piece had become more specialized. At either end of the piece there appeared a prominent outgrowth, the one at the posterior end curving upward and forward. There were two large cavities lined with a single layer of much flattened endothelial cells. These cavities were much larger than any spaces in the original piece of the embryo, and not improbably represent closed and distended blood vessels. The notochord showed at each end an extension of cells which suggested that the organ was undergoing regeneration in both directions. These extensions lay entirely within the new outgrowths from the cut ends of the piece.

Fig. 3 represents a developed fragment taken five days previously from near the middle of an embryo. This piece contained some entoderm which is included in the dark pigmented mass

near the center. At one side there was a thin vesicle filled with fluid. The ectoderm cells covering this vesicle were much flattened. Ten days later the whole piece was larger, owing to the increase in the size of the hollow vesicle which had come to

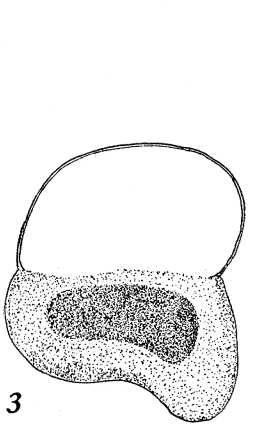

FIG. 3.

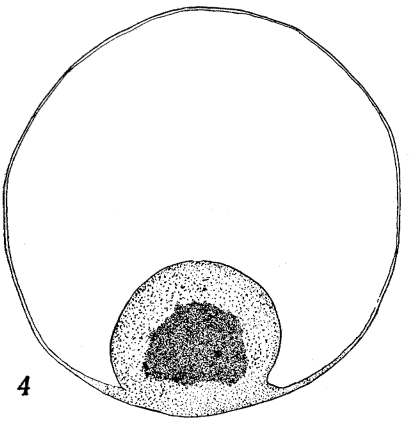

FIG. 4.

FIG. 3. A piece which developed from a fragment of a middle portion of a frog embryo.

FIG. 4. The same piece as is shown in figure 3, but drawn ten days later.

surround most of the rest of the fragment (Fig. 4). The pigmented mass and the tissue around it had assumed a more nearly spherical form, and the whole structure had become more nearly spherical also. Apparently the chief factor in the form changes in this case was the absorption of fluid which caused the increasing distension of the thin walled vesicle. A similar process may account for the large spaces in the fragment shown in Fig. 2. In transferring the nearly spherical fragment to fresh lymph the thin wall of the vesicle partly collapsed, but it was subsequently distended to its previous form. During the time the piece was kept alive, which was about five weeks, it showed no other marked changes in form.

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